HYDROPHOBIC AND HYDROPHILIC DRUG LOADING CAPACITY OF MICRO DIATOM FRUSTULE FROM DIATOMITE

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ABSTRACT

The aim of this work was to strengthen the evidence of using micro diatom frustule as a promising candidate for drug loading materials for both hydrophobic and hydrophilic drug models. The morphological, surface elemental composition of diatomite powder, a raw source of micro diatom frustules and purified diatomite to collect micro diatom frustule were investigated. Scanning electron microscope (SEM) and X-ray photoelectron spectroscopy (XPS) confirmed again the porous silica structure of micro diatom structure as well as validated a necessity of raw diatomite purification before using. UV- vis was used to measure drug loading content of untreated and treated surface of micro diatom frustule with maximum loading for hydrophobic and hydrophilic drugs after 24 hours were at 5.48 ± 0.42% and 5.70 ± 0.34, respectively. Moreover, we also proved that the ability of drug adsorption on materials surface by the reduction of specific surface area and pore size of micro diatom frustule after loading using a (Brunauer–Emmett–Teller) BET method. Besides, the hydrophobic loading capacity of materials was affected by surface modification. Based on the results, micro diatom frustule showed a potential for a drug delivery system.

Keywords: *diatomite; micro diatom frustule; porous structure; drug loading capacity; hydrophobic and hydrophilic drugs.*

1. INTRODUCTION

Drug delivery systems (DDS) have been rapidly developing with both natural and synthetic materials in recent decades [1]. Several materials including synthetic polymers, microcapsules, micelles, micro-or-nanoparticles, and so on have been extensively investigated and applied for drug delivery applications [2]. The most important key to design and select materials for DDS is an efficacy therapeutic agent to target objects. Besides, large specific surface area and biocompatibility have been considered as significant properties of materials for DDS [3].

Amongst numerous materials proposed for DDS, amorphous porous silica have been taken into consideration as a prospect materials classification since they possess thermal stability, high porosity, controllable surface chemical properties and good biocompatibility [4]. Nevertheless, most porous silica has been synthesized from precursors. Thus, these synthesized processes spend time, cost-consuming and toxic waste during the synthesis. Conversely, nature provides some elegant porous biosilica resulting from self-assembly of biomolecules in rich soluble silica environments [5]. Diatom cell wall is an outstanding example of this phenomenon.

Diatomite is a marine sediment, which results from accumulation of dead diatom cells. Main composition of diatomite is the high content of amorphous silica (SiO_2); moreover, diatomite is a high porous material due to the porous structure of the diatom cell wall of diatom frustule. In particular, diatom frustule varies in morphology depending on its original formation, but it presents honeycomb structure of pore size ranging from nano to microscale [6]. This structure is significantly prospective for DDS applications. In addition, diatom frustules

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from diatomite are low cost owing to abundant source.

Indeed, previous studies have taken an interest in application of diatom frustule as a promising candidate for DDS [7], [8]. However, the application of diatom frustule from diatomite as a DDS has some disadvantages such as distinct efficacy of drug loading due to different morphology and surface properties of diatom frustules and contamination contained in a raw material.

In this work, raw diatomite was characterized by its properties in terms of chemical compositions and morphology and separated collect micro diatom frustules before drug-loading experiment. Doxorubicin and ibuprofen were used as hydrophilic and hydrophobic drug models to evaluate drug loading capacity of micro diatom frustule, respectively. Finally, quantitative doxorubicin and ibuprofen loaded by material were measured by ultraviolet-visible (UV-Vis) spectroscopy and change of specific surface area of material.

2. MATERIALS AND METHOD

2.1 Materials

Raw diatomite material was provided by Phu Yen Mineral Joint Stock Company (Phu Yen province, Viet Nam) with white powder form. Micro diatom frustules were collected from raw diatomite powder, as reported in the literature [9]. 3□glycidyloxypropyl trimethoxy silane (GPTMS), doxorubicin and Ibuprofen drug was supplied by Sigma - Aldrich Chemie GmbH (Germany). Other reagents were reagent grade.

2.2 Method

In brief, raw diatomite was purified to eliminate contamination of these materials by treating HCl 1M at 55°C for 24 hours with concentration of 100 mg of powder per ml solution. After acidic treatment, suspension was filtered, and the remaining diatomite containing micro diatom frustule was washed and separated several times in distilled water to remove any trace of acid before drying at 110°C for 8 hours to obtain micro diatom frustule. Afterwards 20 mg of dried micro diatom frustule were suspended in 100 ml of a solution of toluene containing 10% by vol. of GPTMS for 24 hours at RT, followed by filtering to collect modified micro diatom frustule. Then, powder of modified micro diatom frustules was twice rinsed with isopropanol before drying in a desiccator at RT.

For ibuprofen loading, 80 mg of obtained material was added to 2.5ml of ibuprofen solution in ethanol at 5mg/ml. Subsequently, the suspension was incubated with slowly shaking for 4, 6 and 24 and 72 hours, then extracted supernatant to measure residual concentration of ibuprofen in ethanol.

In case of doxorubicin loading, modified micro diatom frustule was first suspended in distilled water at concentration of 2.5 mg/ml. Then, 100 µl of doxorubicin at 1mg/ml in DI water was added into 500 µl of diatom frustule suspension, followed by modifying the pH experimental conditions of suspension of drug-material at the value of 7.2, 3.9 and 8.6, respectively. The suspension was shaken at 500 rpm for 24 hours at RT. After incubation, the supernatant of suspension was taken to measure drug residual.

2.3 Characterization

Surface chemical composition of raw diatomite, micro diatom frustule structure was characterized by X_ray photoelectron spectroscopy (XPS) with a Scienta Gammadata ESCA 200 (Sweden) whereas scanning electron microscopy (SEM, FESEM-Supra 40, Zeiss, Germany) was used to monitor morphology and particle size of these materials.

The concentration of ibuprofen and doxorubicin content in supernatant after loading was quantified using a Nanodrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, UK) at 264nm and 500nm, respectively. The drug loading content was calculated from the equation.

Drug loading content (%) = (weight of drug in material/ material weight) · 100%

Specific surface area of material before and after doxorubicin loading for 24 hours was determined using a Nova Station A (Quantachrome –US).

3. RESULTS AND DISCUSSION

3.1 Materials characterization

SEM images of raw diatomite powder and materials after removing contamination are shown in the figure 1a) and b).

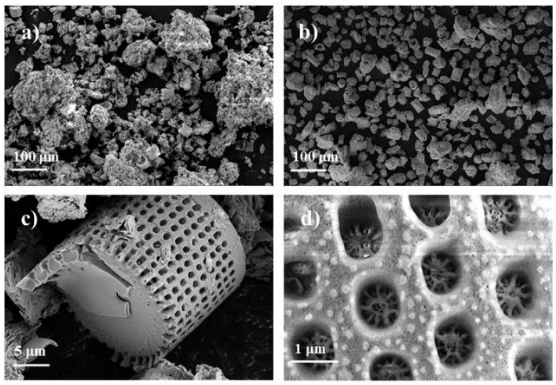

Figure 1*. SEM images of raw diatomite (a), micro diatom frustule after purification (b), diatom frustule morphology (c) and pore structure of diatom frustule wall (d).*

SEM analysis reveals a wide range of particle size in raw materials. In particular, particle size could range from 20 ⌠m to 200 ⌠m. Moreover, small particles present fragments of broken diatom frustules whilst large particles result from fragment aggregation and possibly contaminated components. In addition, just a few full diatom frustules are observed in the scanning area. On the contrary, SEM image (figure 1b) of material after purification shows a narrow particle size distribution although aggregation of fragments still remains in the product. Small fragments as well as large aggregation in materials significantly decrease. Meanwhile, material after purification obtains more whole diatom frustules.

Figure 1c) and d) demonstrate morphology of diatom frustule that have the typical honeycomb open-ends cylindrical shape, with a diameter of 15 – 20 µm and length of 20–40 µm, and a dense network of micropores about 1 ⌠m in diameter containing nano-pores with diameters in the range of 200nm to 800 nm. The typical structure assigns them to Aulacoseira species [10], which is suitable for DDS [7].

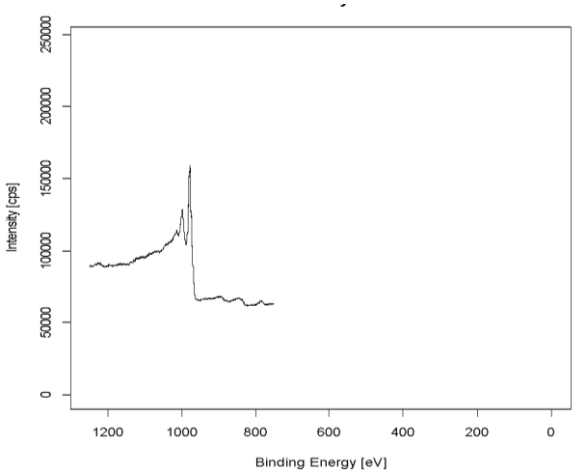

Figure 2*. Typical XPS survey spectra of materials*

The XPS survey spectra of surface chemical composition of materials obtained by XPS is shown in figure 2. Based on binding energy, XPS spectra identifies the material surface formed of a rich list of elements. In association with figure 3 and table 1, elemental composition of material surface before and after purification consists mainly of silicon, oxygen, carbon and aluminum.

Besides, other elements such as magnesium, iron, potassium (full data not shown) exist in materials with lower content. The presence of high carbon and aluminum in the raw material can be explained by an existence of organic contamination and silicate due to formation of diatomite [11].

Table 1*. Main atomic composition quantified by XPS spectra of diatom frustule surface before and after purification*

Sample	Si2p	O1s	C1s	Al2p
RD	12.80	60.85	20.30	1.82
PD	18.98	63.14	9.06	4.55

RD: raw diatomite powder; PD: micro diatom frustule powder after purification.

After purification, it is obviously clear to observe a strong reduction of C1s peak

intensity (figure 3) that refers to a drastic decrease of around 10% of carbon content in materials, as shown in table 1. In the meanwhile, an increase of silicon, oxygen and aluminum content in purified materials results in carbon decrease (table 1). A significant decrease of carbon content can be due to reduction of organic contamination.

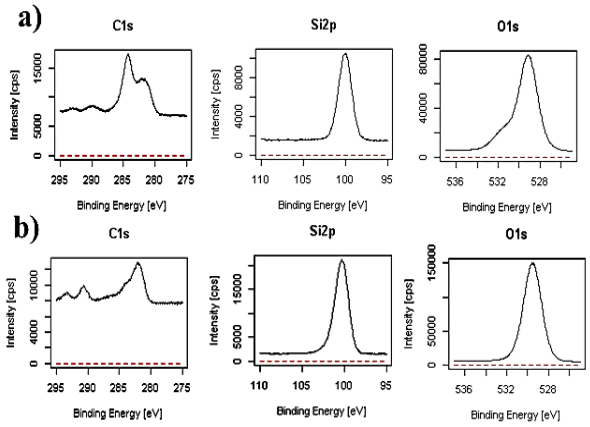

Figure 3. *Binding energy of C1s, Si2p, O1s core levels surveyed by XP for a) raw diatomite powder and b)micro diatom frustule powder*

However, carbon and a part of aluminum remaining in the material after purification may also relate to organic association and incorporate trace elements during self-assembly of diatom frustule [12].

Both SEM observation and XPS analysis present the efficiency of the purification step, which is a necessary stage for materials used for DDS.

3.2 Hydrophobic and hydrophilic drug loading capacity

3.2.1 Visualization of drug loading of micro diatom frustule

Figure 4 illustrates photos taken during micro diatom frustule loading doxorubicin at the beginning and end of the process. It is clear that the color of doxorubicin – micro diatom frustule suspension completely changes. In detail, suspension has crimson color at t=0 time point, but it transfers into light pink color after 24 hours of loading process. This phenomenon can indicate drug loading ability of material.

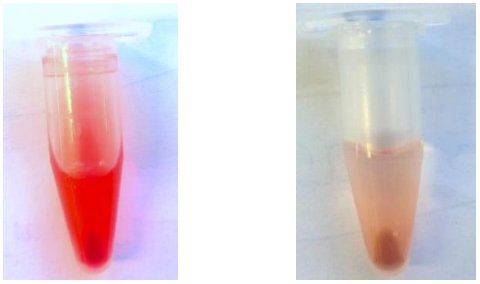

Figure 4. *Optical images of suspension of doxorubicin and materials a) at t=0, and b) t= 24 hours*

3.2.2 Ibuprofen loading of micro diatom frustule

The results of drug loading content in micro diatom frustules powder and the powder treated with GPTMS are presented in figure 5. As expected, ibuprofen loading in both MDF and MDF-GPTMS progressively increased after 24 hours of incubation until 24 hours, then decreasing until 72 hours. The decreased amount of drug containing the materials can be explained by the release of drug into aqueous environment again due to the diffusive phenomenon of drug–surface interaction [13].

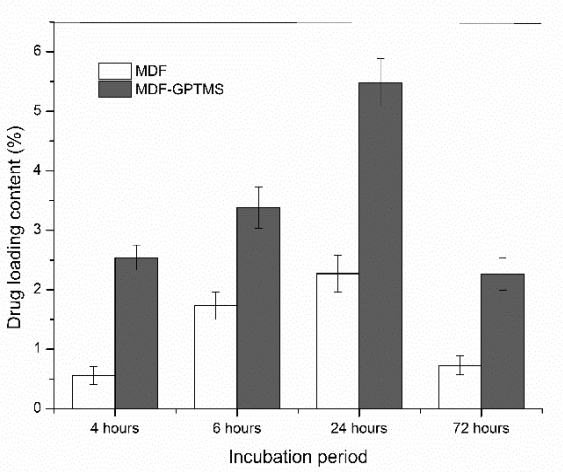

Figure 5. *Ibuprofen drug loading of micro diatom frustule (MDF) and micro diatom frustule modified surface with 3−glycidyloxypropyl trimethoxy silane (MDF-GPTMS) during 4,6, 24 and 72 hours*

Surface modification with GPTMS improved the loading efficiency of ibuprofen with respect to the untreated material, with a

drug content that doubles after 24 hours of incubation passing from 2.27% to 5.48%, much higher than for the untreated samples (MDF). These results are in agreement with the previous finding about the effects of agents based on silane on drug loading capacity of diatom [14].

3.2.3 Doxorubicin loading

A Doxorubicin loading experiment of micro diatom frustules was designed following the period, in which the material contained a maximum amount of ibuprofen.

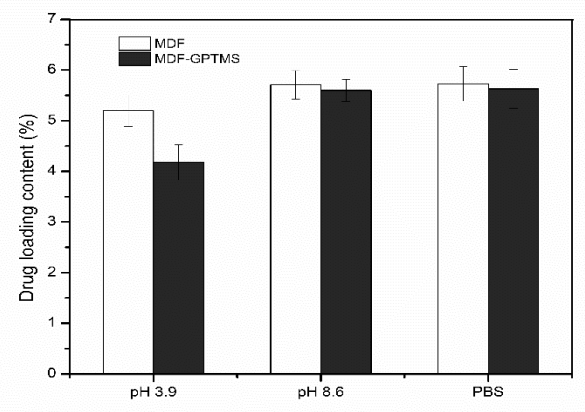

***Figure 6**. DoxorubiciN drug loading of micro diatom frustule (MDF) and micro diatom frustule modified surface with 3□glycidyloxypropyl trimethoxy silane (MDF-GPTMS) in different pH conditions.*

Quantitative doxorubicin loaded by the materials after 24 hours of incubation is shown in the figure 6. In general, the loading of the hydrophilic doxorubicin is almost unaffected by the surface modification at higher pH, with a decrease of the loading in acidic condition. Particularly, the doxorubicin loading content ranged from 5.2 to 5.7%, except for those modified by GPTMS in an acidic environment.

The lower value of drug loading content for the material treated in acidic condition may be related to the dissolution in acid of the silane group, preventing adsorption of doxorubicin into material surface [15].

3.2.4 BET surface area results

BET specific surface area and pore size of materials before and after doxorubicin loading for 24 hours are demonstrated on figure 6 and table 2.

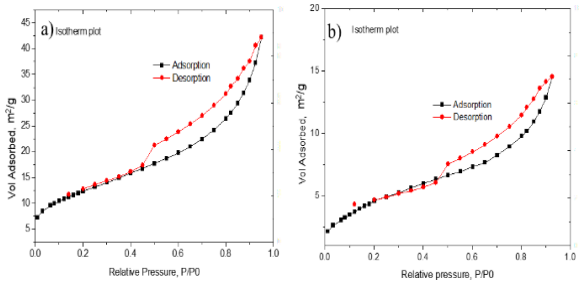

***Figure 7.** Nitrogen adsorption and desorption isotherms before (a) and after (b) drug loading (doxorubicin)*

BET results indicated that the specific surface area and pore volume of materials after loading with doxorubicin strongly decreased by 62.6%. The pore diameter of the diatom skeleton also decreased from 131 to 127 nm.

***Table 2**. Specific surface area and pore size before and after doxorubicin loading*

Sample	S_{BET}, m^2/g	Pore size, nm
MDF	42.74	131
MDF+drug	15.96	127

The reduction of specific surface area and pore size after doxorubicin loading refers to efficiency of adsorption of drug in the surface of diatom frustule as well as round the pore located on the wall of diatom frustule. This phenomenon may be concerned with the fast release of drugs after the loading process.

4 CONCLUSION

In this study, we want to highlight the workable application of a natural porous silica from diatomite as potential drug cargo.

The structure and properties of micro diatom frustule were characterized by advanced techniques including SEM, XPS to prove the absorption and penetration on diatom surface, inside pores. However, the efficiency of drug loading has been influenced by the isolating process.

The results also confirmed that surface modification of the diatom skeleton by

GPTMS positively impacts on the drug loading capacity of hydrophobic ibuprofen, having a negligible or no effect on the loading of the hydrophilic doxorubicin.

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